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EMBRYO DEVELOPMENT AND POLYEMBRYONY IN RELATION TO THE PHYLOGENY OF CONIFERS[1]

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A consideration of embryogeny has played an important part in nearly all discussions of phylogeny, but the embryo development of conifers has offered so many variations and apparent anomalies that many students of Gymnosperms have been in doubt as to whether the embryogeny should be considered very seriously in connection with a study of the morphology of this group. So far as I know, the question of polyembryony has not generally entered into these comparisons, but has been looked upon as an extremely variable feature and one of little morphological importance. The present discussion is an attempt to take into account both the embryo development and polyembryony in making comparisons between the embryogenies of conifers, and aims to point out how these features of embryo development may prove to be very valuable criteria in arriving at the true phylogeny of the Coniferales.

Since my new interpretation of the embryogeny of conifers involves an accurate knowledge of the condition known as polyembryony, it will be necessary to consider briefly the events of proembryonic development that lead up to and accompany this condition. We will use Pinus as our first illustration, because its better known details of development serve as an excellent standard of comparison for the other conifers. I expect to show, further, that the embryogeny of Pinus occupies a very primitive position in relation to all conifers whose embryogeny has thus far been described.

PINUS

In the proembryo stage of Pinus it is well known that walls appear following the mitosis between the four- and the eight-nucleate stages, but it should perhaps be emphasized that after these first walls are formed (fig. 2), the cells of the lowest tier (*p*) are the initial cells which give rise to the four distinct embryos that completely separate from each other. Another free nuclear division occurs in the tier of incompletely walled cells above

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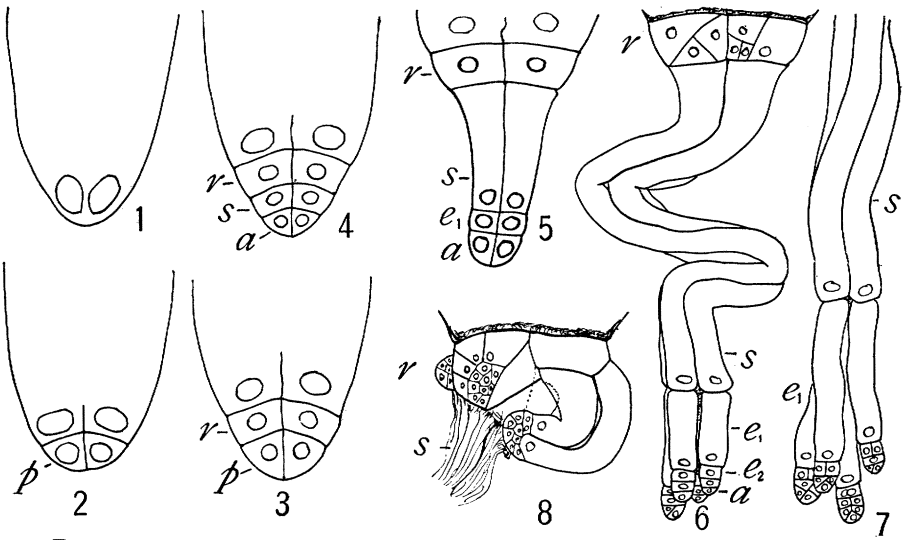
this, and hence the tier of cells *r* (fig. 3), the rosette tier, is also organized directly after a free nuclear division in the proembryo. These rosette cells are now known to be embryo initials (2).

In this stage, shown in figure 3, with the upper aborting tier of four free nuclei and with eight-walled cells beneath them arranged in two tiers, we have the initial cells of all the embryos that are regularly produced



FIGS. 1–8. Embryogeny of Pinus. FIGS. 1–4. Sectional views of proembryonic stages. FIG. 5. Embryos when suspensors begin elongating. FIG. 6. Separation of four embryos coming from *p*. Embryos arising from rosette (*r*) are shown in figure 8. *a*, apical cell; *s*, suspensor; e_1, e_2, embryonal tubes that add to suspensor; *p*, tier of initial cells of primary embryos; *r*, rosette tier.

from a single fertilized egg in the pine. Of course, some of these initials, especially those of the rosette group, may abort in this stage or in any subsequent stage of development.

The embryos that arise from each of these initial cells begin their development by true apical cell growth; first the apical cell has only one cutting face, and later, when the embryos have separated, it has three cutting faces. The apical cell vanishes, usually before an embryo of more than 500 cells is formed.

Following the organization of these eight walled cells, the embryo initials of the tier *p* undergo simultaneous division in which the tier *s* is formed (fig. 4). These first segments (*s*) of their respective apical cells elongate to form suspensor cells (figs. 5, 6), while the apical cells (*a*) form additional segments, e_1, e_2, etc., that elongate and add to the suspensor.

By this time the four vertical rows of cells, representing as many embryos, have separated, and the rosette cells (*r*) begin to proliferate to form the rosette embryos. The four rosette embryos usually do not actually

separate (fig. 8), but are nevertheless entirely independent of each other in their further development, while the four primary embryos completely separate from each other, eight embryos being the normal product of each egg in Pinus.[2]

Cleavage Polyembryony

This separation of the zygote into a number of smaller units which undergo competition with each other is called cleavage polyembryony, to distinguish it from the simple polyembryony that may result from the fertilization of several eggs. The free nuclear divisions occurring during the proembryo stage in Pinus are followed by the equal cleavages which organize the initial cells of each of the embryos that separate. It is well known that only one of these many embryos, produced either by cleavage polyembryony or by simple polyembryony survives to the maturity of the seed. This is surely a "survival of the fittest" if vigor in the embryo is any sort of measure of future fitness.

This cleavage polyembryony with very definite proembryonic organization, which is present in Pinus, is apparently a primitive character in the development of this group of seed plants. This character tends to be modified or eliminated, reverting to the condition of simple polyembryony, as we advance along several phylogenetic lines, and is lost by the time the level of the Angiosperms is reached. If a splitting of the embryo is found among Angiosperms, which is rare, it is a cenogenetic character, a condition which has not necessarily been carried over from Gymnosperms.

It is well known that the term polyembryony when applied to Angiosperms has no such definite meaning as when applied to Gymnosperms, for among conifers the term has been used to designate the plurality of embryos which arise from the cleavage of one egg (cleavage polyembryony), or from the fertilization of several eggs (simple polyembryony), but not otherwise. Among Angiosperms, as summarized by Coulter and Chamberlain (12), the embryos are known to arise from a plurality of eggs in only two species (widely separated in phylogeny), occasionally by a process of budding from the suspensor, and in only one species by the splitting of an embryo derived from the single egg. Furthermore, the extra embryos are derived from synergids in about twelve species, from nucellar or other tissue outside of the embryo sac in eleven or more species, from antipodal cells and even from endosperm in others, while false polyembryony may occur through the fusion of ovules, etc. Clearly this Angiosperm polyembryony has little in common with the phenomenon when found in Gymnosperms.

In all Gymnosperms, polyembryony results in a selection of a single embryo from a larger number, long before the seed is matured, while in Angiosperms it is a common thing to find that several embryos survive to the maturity of the seed. The suspensor of the Gymnosperm embryo

[2] A more detailed review and discussion of the proembryonic development of Abietineae is given in another paper by the author (4).

is an organ of competition, the structure upon whose merit the selection of the surviving embryo depends, while in Angiosperms it is usually a chain of comparatively unelongated cells which carries the embryo into the central portion of a very soft endosperm. Ginkgo, which has a relatively short suspensor, seems to be about the only Gymnosperm which occasionally matures more than one of its several embryos, and even here Cook (11) found this occurring in only two percent of the seeds. It is much more infrequent than this in Pinus, and probably in all other Gymnosperms. It is clear that the erratic character of polyembryony in Angiosperms should not influence our judgment in deciding on the phylogenetic value of this feature in Gymnosperms.

Simple polyembryony is found among cycads, since these have constantly several archegonia; but none of them are known to possess cleavage polyembryony with its accompanying phenomena, so that polyembryony is a feature too uniform to be of value in showing the affinities of the genera within the Cycadales. However, the ordinary anatomical characters of the proembryo have been used very effectively for this purpose by Chamberlain (6).

Among conifers, much more precise comparisons of embryonic development are possible when we once understand these greater variations of the proembryo and of the early embryo brought about by cleavage polyembryony; stages of development which one would otherwise expect to find rather conservative and uniform. The peculiar splitting of the embryo, which was introduced somewhere in the ancestry of Pinus, has persisted for some time in the evolution of the conifers, and was suppressed or eliminated by a number of distinct methods. The rosette embryos, rosette cells that abort, and many other early embryo features are only results of cleavage polyembryony. Together with the apical cell (doubtless a Pteridophyte character which has persisted), these features give us a splendid array of consistent characters to serve as an index to the natural classification of the groups.

Abietineae

In a very recent paper on "Polyembryony among Abietineae" (4), I have made a number of comparisons of the embryos of this group, which are summarized with greater accuracy in the diagram of figure 9. While the proembryos of all Abietineae (with the possible exception of Pseudotsuga) appear to be identical (4), the condition of cleavage polyembryony (*Cl.p*) is restricted to Pinus, Cedrus, and Tusga. *Abies balsamea* displays an occasional embryo with cleavage polyembryony, but normally it has only simple polyembryony and is similar to Larix, Picea, and Pseudotsuga in this respect.

It will be seen that the apical cell (*A*) has the same range of distribution within this group. Fusion of embryos seems to be the method by which

both of these characters disappeared from the ontogeny of the higher Abietineae.

Rosette embryos (*R-em*) usually develop in Pinus, are somewhat less developed in Cedrus, while Abies has rosette embryos only rarely, about

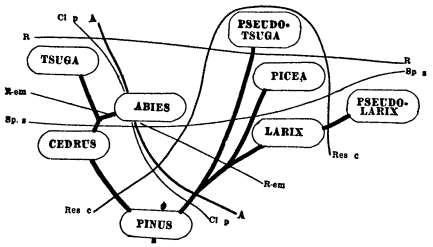


FIG. 9. Diagram to show the affinities of the genera of Abietineae, based on embryogeny. *A*, probable range of apical cell; *Cl.p*, range of cleavage polyembryony; *R-em*, range of rosette cells which may give rise to rosette embryos; *R*, range of abortive rosette cells. Two anatomical characters are included: *Res.c*, the range of resin canals in secondary wood, defining the Pinae of Jeffrey (19, 20); *Sp.s*, range of spur shoot, used by Engler and Gilg (16) and others in subdividing the Abietineae.

as often as it has cleavage polyembryony. However, these two occasional characters of Abies are not necessarily associated with each other in their occurrence.

In Picea, Larix, and *Abies balsamea* (normally) the rosette embryos are represented only by their initial cells (*R*). Tsuga also has only these aborting rosette cells, though its primary embryos separate as in Pinus. These abortive rosette cells are the last rudimentary structures that remain to indicate the origin of these embryos with simple polyembryony from the type of cleavage polyembryony found in Pinus.

One of the most formidable obstacles to the opposite interpretation, namely, that the embryo of the Picea or Larix type has given rise to the pine type with its cleavage polyembryony, comes from the study of these rosette cells. It is impossible for these abortive structures as represented in Picea or Larix to have given rise to the active rosette embryos of Pinus and Cedrus. On the other hand, the view that rosette embryos and the abortive rosette cells are steps in the elimination of cleavage polyembryony offers a very satisfactory hypothesis that is consistent with the facts and that very definitely points out the direction of this polyembryonous evolution.

In Pseudolarix the rosette cells are present (29), but nothing has been described or figured to indicate whether cleavage polyembryony or rosette embryos develop at a later stage. The exact position of Pseudolarix is still doubtful, but from what is known of its embryo, and considering that it resembles Larix in its spur shoot, it is safest to place it in the diagram near Larix.

In Pseudotsuga, the uppermost walled cells of the proembryo elongate to form the suspensor, and therefore no aborting rosette cells are found.

It is interesting to note how these embryo characters relate themselves to the results of the anatomists. The distribution of the resin canal characters described by Jeffrey (19, 20) are included in the diagram (*Res.c*), cutting Pinus off from Cedrus, although these two genera are very similar on the basis of embryongeny and also in the spur shoot characters used by Engler and Gilg (16) and others as a basis for subdividing the Abietineae. On the other hand, the distribution of the apical cell, cleavage polyembryony, and rosette embryos cuts Pinus off from the rest of the Pinae of Jeffrey. The intermediate position of Cedrus between Pinus and Abies was pointed out by Jeffrey (19) and by Chrysler (7) on the basis of medullary rays. Pinus has such an array of primitive features, both embryological and anatomical, that there can no longer be much doubt of its primitive position in any natural phylogeny of Abietineae.

Other Coniferales.

In the light of the foregoing, let us now consider the affinities of the other Coniferales as revealed by their embryo development, where this is sufficiently known. The interpretations which I shall give, while not generally those given by the investigators to whom the particular work is credited, do no violence to the facts as they have been described, and for any errors due to these interpretations I assume full responsibility.

I will first present the line of evolution between Pinus and Araucaria, based on embryogeny. There are not many known types that fit in between these two very different methods of embryo development, but we have steps enough to give us a definite clue to the possible lines of advance, and to suggest the kind of embryogeny from which that of the araucarians was derived.

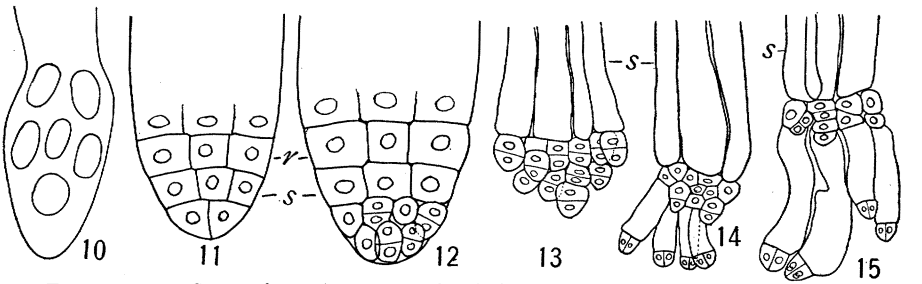
The number of free nuclear divisions is increased and a larger number of embryo initials is formed, resulting for a time in greater cleavage polyembryony. Advancing a little further, we find that only a small portion of these potential embryo initials function. Cleavage polyembryony is then eliminated by the modification of the terminal portion, which organizes a cap and prevents the embryos from splitting apart. The apical cell also persists for a time in this line of evolution, and it appears that the formation of this cap over the advancing end of the embryo is the thing which does away with the apical cell stage. Here again we have probably had the apical cell stage and cleavage polyembryony eliminated by the same device.

Even if we take the position that Pinus did not directly give rise to araucarians, it is apparent that they had a common origin and were derived from a condition of cleavage polyembryony. Pinus has remained in this condition with very little modification, while the araucarian embryogeny has become specialized, and has completely eliminated cleavage polyembry-

ony. A few stages in this araucarian line of evolution may be illustrated by Sciadopitys and the podocarps.

SCIADOPITYS

The embryogeny of Sciadopitys (figs. 10–15) has been partially described by Lawson (27) and Arnoldi (1), and represents a step in the direction of Araucaria. There are at least eight free nuclei before walls form (see fig. 10), and Lawson states that "eventually the proembryo consists of three tiers of cells and one tier of free nuclei," from which I have supplied fig. 11. However, the organization of embryo initials continues in the terminal tier



FIGS. 10–15. Stages in embryogeny of Sciadopitys. Figure 10 after Lawso n (27) figures 11 and 12 supplied from description by Lawson; figures 13–15 after Arnoldi (1).

until a group of about 16 cells is formed (fig. 12). (This stage is also supplied from Lawson's description and from a study of the next stage (fig. 13) by Arnoldi.) Finally the suspensor tier elongates and thrusts the terminal group of cells into the gametophyte, a stage shown in figure 13. That the cells of this terminal group are really embryo initials, mostly advanced to the two-celled stage, is borne out by the next two figures.

It will be noted that in Sciadopitys a large number of embryo initials are produced, and the functional ones are organized in this instance *after* the first walls have formed in the proembryo; that is, the lowest functional group is not the first group of embryo initials to organize, as in Pinus, but the last. I would look upon a late organization of the functional embryo initials from previously walled cells as a more advanced condition, for this occurs quite generally in the more recent conifers.

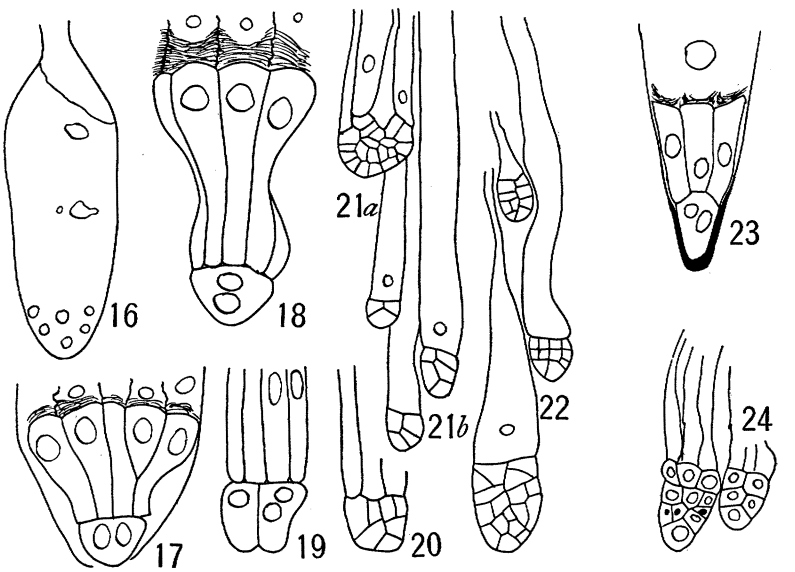
Rosette embryos undoubtedly exist in Sciadopitys, as Lawson accounts for walled cells above the suspensor, and Arnoldi definitely described a group of proliferating cells occupying this position which he noted as a possible vestigial protocorm, comparable to the protocorm of lycopods. He states: "Ich kann gewiss nicht bestimmt entscheiden, ob es ein morphologisches Gebilde ist, oder eine Anpassung zur noch weiteren Polyembryonie." Unfortunately Arnoldi gave us no figures of this "protocorm," but it is probably safe to infer that he saw a group of rosette embryos, and that the entire embryogeny of Sciadopitys represents a modification in the direction of greater cleavage polyembryony.

PODOCARPINEAE

The embryo development of the podocarps, according to the meager descriptions available, represents a condition much further removed from Pinus, one which is to be looked upon as modified from a form intermediate between Sciadopitys and the araucarians. In many respects, these podocarp embryos illustrate some of the general tendencies of embryo modification which brought about the highly specialized araucarian type, and are therefore considered here.

The proembryo of Podocarpus (fig. 17) consists of two very unequal tiers of cells, and some free nuclei in an open tier above. Figure 18 shows an early proembryo with suspensors elongating, and with its binucleate terminal cell undergoing further division in figure 19 before the embryo initials, which may separate, are formed. From the fact that these suspensor cells in this case were organized from free nuclei, one could infer that these are suspensor-forming embryo initials. The rosette cells of Pinus, which certainly are embryo initials, were sometimes found to elongate as suspensors (2), and we might expect to find some groups of conifers in which such unfavorably placed embryo initials are normally modified to form suspensors.

While *Podocarpus coriaceus* (figs. 16–22), which was investigated by Coker (8), produced walls only after sixteen or more free nuclei were formed,



FIGS. 16–24. Stages in embryogeny of *Podocarpus coriaceus*, after Coker (8). FIG. 23. *Podocarpus nivalis*, showing binucleate terminal cell enclosed by a thick cellulose cap. FIG. 24. Embryos of *P. ferrugineus*. Apical cell stage appears to exist in figures 20 and 21*b*–24. Figures 23 and 24 after photomicrographs by Sinnott (39).

Sinnott (39) described *P. totara* and *P. nivale* as producing walls at an earlier stage, which fact places the Podocarpineae a little closer to the Abietineae.

Coker states that either the embryo may split up or its parts may unite in producing the embryo, but it is highly probable that cleavage polyembryony is the normal condition in the species which he investigated. All his figures of embryos that presumably were produced by simple polyembryony appear very much like those which one would expect to see in the older separated embryos which have a secondary suspensor, and we know that all conifers produce such secondary additions to the suspensor by the elongation of cells to form embryonal tubes. From the interlocking articulation of the embryo of figure 21*a* with its suspensor, it is evident that these are embryonal tubes and not the primary suspensor, as inferred by Coker; but the largest embryo of this group is not the terminal one in this particular instance.

It is also possible that the apical cell stage is found in these separating embryos of Podocarpus, as may be seen by a careful study of figures 21–24. No rosette cells are found in the above described species (I refer to walled rosette cells, not the free nuclei that abort above).

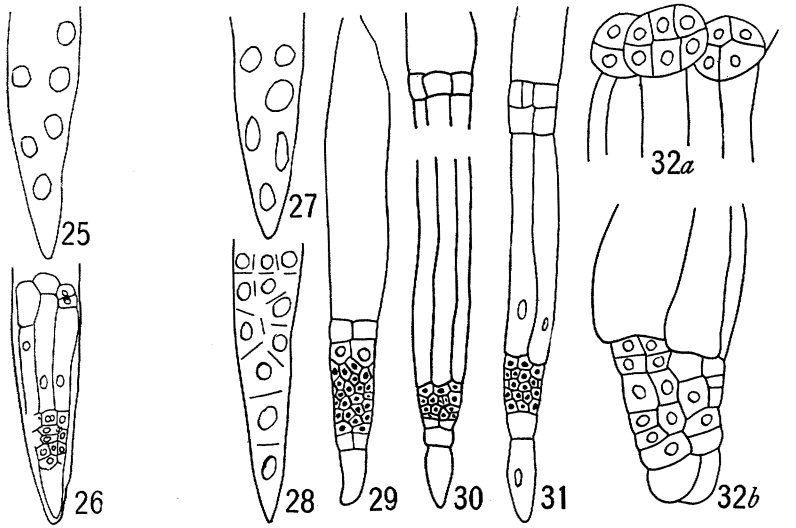
In *Podocarpus nivalis*, Sinnott described and figured a thick cellulose cap "protecting" the binucleate terminal cell, as shown in figure 23, which is pushed far into the gametophyte before it divides further, and "instances of budding or of single suspensors giving rise to embryos were only rarely observed." Does it not appear that this thick cellulose cap would tend to prevent a splitting of the embryo and so to cause the initial cells of the lower tier to combine and produce a single embryo?

I have occasionally observed a cap of wall thickening which appeared to hold together the terminal cells in the embryo of Pseudotsuga,[3] but this structure is not found in the slightly later stages. It is evident that cleavage polyembryony occurs only with considerable difficulty where such a thick cellulose cap protects the terminal group of cells. I am inclined to look upon these caps as mechanical devices which prevent cleavage polyembryony.

Another type of embryo development, probably a higher specialization of that above described, is also found in the Podocarpineae. I refer for illustration to the embryo of *Podocarpus spicatus* (figs. 25–26), one of the Stachyocarpus group (39), in which the terminal cells organize into a cap while the cells above the cap give rise to a single embryo and its suspensor of embryonal tubes. In this case we have no cleavage polyembryony, and it appears that the function of this cap, which is soon sloughed off, may be to prevent this splitting of the embryo, a danger present only in the first stages. However, it would probably not prevent cleavage polyembryony as successfully as the more elaborate caps found in Araucaria and Agathis.

[3] Unpublished work.

The Cephalotaxus embryo shown here in figures 28–32 doubtless has been modified from something like this *Podocarpus spicatus* type of embryo. It has, however, a feature which would relate it more directly to Pinus



FIGS. 25–26. Stages in embryogeny of *Podocarpus spicatus*, showing terminal cap cell, suspensor cells beginning to elongate, and the group of embryo-forming cells between. After photomicrographs by Sinnott (39). FIGS. 27–32. Stages in embryogeny of Cephalotaxus, showing terminal group of cap cells, embryo-forming cells, suspensor, and what is probably a group of rosette cells in 32*a*. FIG. 27. *C. drupacea*, after Lawson (25). FIGS. 28–32. *C. Fortunei;* figure 28 after Coker (10), and figures 29–32 after Strasburger (41).

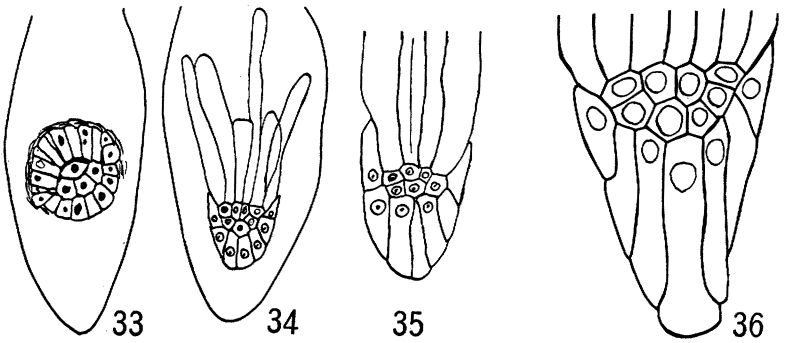
namely, a conspicuous group of rosette embryos (fig. 32*a*) above the suspensor. The figures here shown are taken from Strasburger (41), who delineated this feature without comment. A similar condition of the rosette may yet be found in the podocarps, for it seems to be suggested by figure 26.

The group of cap cells in this case is probably also a device to prevent cleavage of polyembryony or apical cell growth, for this cap is also sloughed off (fig. 32*b*) soon after the proembryo stage, the stage in which cleavage polyembryony is found, if at all.

ARAUCARINEAE

While I would not derive the araucarian type of embryo with its elaborate cap (figs. 33–36) from these podocarp embryos, it is probable that these two groups had a common origin, that the caps of the embryos of both were derived in response to similar conditions, and that these peculiar structures in both instances serve to prevent cleavage polyembryony. The increase in the number of free nuclear divisions in the proembryos of both these groups may be due to a past history of greater cleavage polyembryony, which might well have been overcome by a mechanical device.

In the Podocarpineae and Cephalotaxus, the functional initials were reduced by the abortion of a large portion of the free nuclei in the proembryo, the walled cells below these became suspensor-like, and cleavage poly-



FIGS. 33–35. Stages in embryogeny of *Agathis australis*. FIG. 33. Proembryo after wall formation, × 200. FIG. 34. After cap is formed and suspensor begins to elongate, but before archegonium is filled, × 260. FIG. 35. Section through tip of older embryo showing the small cells below the suspensor from which the embryo is derived, × 460. After photomicrographs by Eames (15). FIG. 36. Embryo of *Araucaria brasiliensis*, drawn to half the scale of figure 35, × 230. After photomicrograph by Burlingame (5).

embryony was eliminated by the organization of caps of various kinds; while in the Araucarineae all the free nuclei are utilized and become organized into an embryo whose terminal portion has a multicellular cap, as shown in figures 33–36.

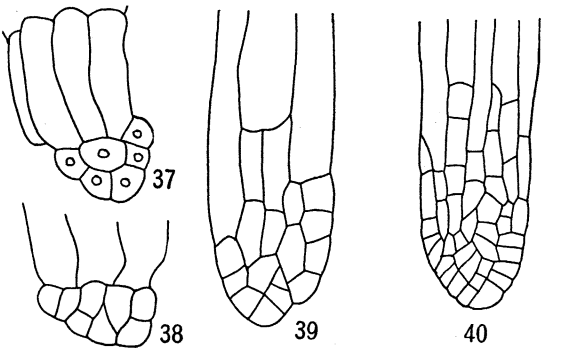
These embryos of Agathis and Araucaria were very satisfactorily described by Eames (15) and Burlingame (5), and neither of these investigators found cleavage polyembryony. In both these species the free nuclear divisions of the proembryo are essentially the same. The proembryo is confined to a samll portion of the egg cytoplasm, and the embryo has begun to elongate considerably before the archegonium is filled by its tissue. The entire embryogeny of the Araucarineae bespeaks a high degree of specialization.

That the cap is not particularly useful in protecting the embryo in its penetration of the gametophytic tissue was brought out by Burlingame (5), and the possibility that it is a special secretive organ is just as remote; but, since other conifers appear to have developed mechanical devices to prevent cleavage polyembryony, an explanation of this nature is possible.

An explanation which would derive the Abietineae from the Aracuarineae on the basis of embryogeny is beset with great difficulty, if not impossible; but it is not so difficult to show how the araucarian embryo has developed from a form very similar to that of Pinus as a specialization along a particular line. At the same time this latter theory offers a satisfactory explanation for the nature of the cap.

TAXADS

The taxad line may have been derived from some of the podocarps, or more probably along with these from a condition nearer to that of Pinus. In Taxus (18) there are at least 16 free nuclei before walls form, and the terminal tier of the proembryo may contain several cells, probably all of



FIGS. 37–40. Stages in embryogeny of *Taxus baccata*, × 125. FIG. 37. Proembryo at beginning of suspensor elongation, after Hofmeister (17). FIG. 38. Slightly later stage. FIGS. 39 and 40. Later stages showing apical cell in early embryo. Figures 38–40, after Strasburger (40).

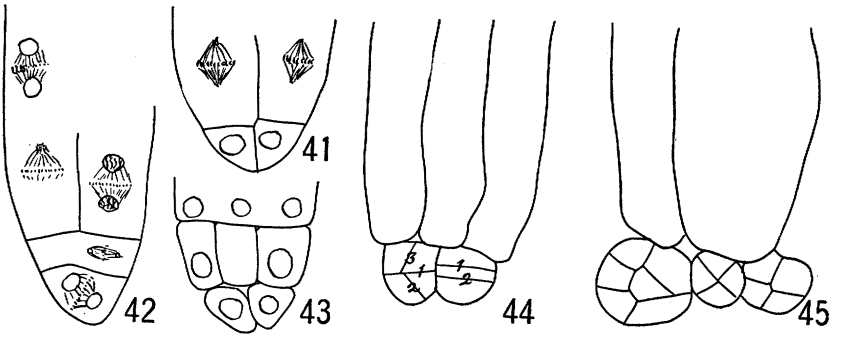
them potential embryo initials. Apparently one of these several cells, doubtless the one most favorably situated, begins to cut off apical cell segments and gains the ascendency over the others (40), which in turn contribute by their activity to the suspensor. Figures 39–40 by Strasburger (40) show that the apical cell persists for some time, and this furnishes an instance of the elimination of cleavage polyembryony with the retention of the apical cell.

According to Jäger (18), some of the upper suspensor cells of Taxus may occasionally break away and appear to give rise to small secondary embryos, but practically always the proembryonic cells all combine into a single embryo. Thus, *Taxus baccata* appears to have practically overcome cleavage polyembryony, but still shows some vestigial evidence of an origin from this condition. In commenting on this Jäger (page 182) makes the significant statement that it appears to him that occasionally all the cells of the early proembryo show a capacity for giving rise to embryos, and in this statement he seems to have approached very close to the present explanation.

In Torreya, the proembryonic tissue fills the entire egg, while in Taxus it is confined to the lower portion of the egg. Torreya has only four free nuclei before walls form, and, though no cleavage polyembryony occurs, the vestigial evidence of it in the form of occasional secondary embryos has been described (14). The differences between Taxus and Torreya may be looked upon as due to higher specialization of the latter, and it is therefore questionable if the apical cell stage exists in Torreya.

TAXODINEAE

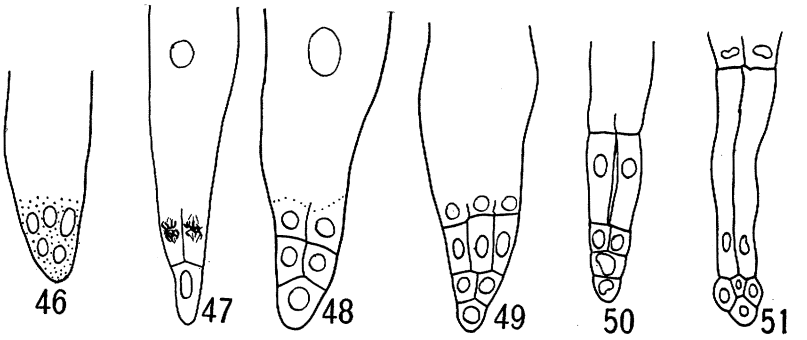
Among Taxodineae, the excessive cleavage polyembryony described for Sciadopitys becomes reduced, as illustrated by Taxodium (9) (figs. 41–45). Figure 43 doubtless represents two unequal tiers of embryo initials in addition to the upper aborting nuclei. The lowest tier forms separate embryos (fig. 44), while the next tier of initials above it forms suspensor



FIGS. 41–45. Stages in embryogeny of *Taxodium distichum*, × 200. After Coker (9).

cells. Cleavage polyembryony is apparent. It is probably the normal condition, and we sometimes have the organization of embryo initials after the first appearance of walls, as shown in figure 42. Coker shows the order in which the walls appear in the embryos of figure 44, which is a good start for an apical cell stage. In general the embryo of Taxodium shows great similarity to that of Pinus, except for the unequal organization of the tiers in the proembryo (see figs. 41 and 43) and the absence of the rosette cells.

Cunninghamia (28) may be a step higher, for here it is highly probable (judging from the few stages described by Miyake (figs. 46–51)) that only the lowest embryo initial gives rise to the embryo. This may have been the method of overcoming cleavage polyembryony in this line of evolution,



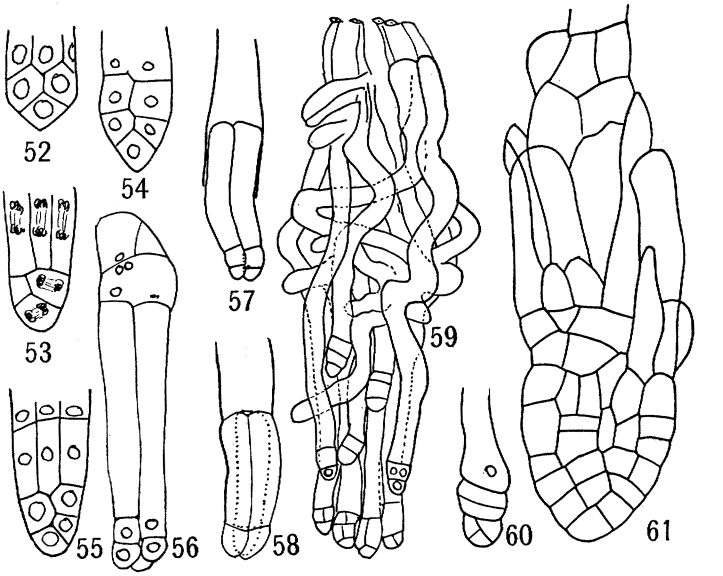
FIGS. 46–51. Stages in embryogeny of *Cunninghamia sinensis*, × 110. After Miyake (28).

the functioning of only the lowest embryo initial, the others giving rise to the suspensor. Whether or not an apical cell organizes in Cunninghamia remains to be discovered.

Cryptomeria (24) belongs somewhere in this group on the basis of embryogeny, but the account and figures given by Lawson are not complete enough to warrant any definite conclusions.

CUPRESSINEAE

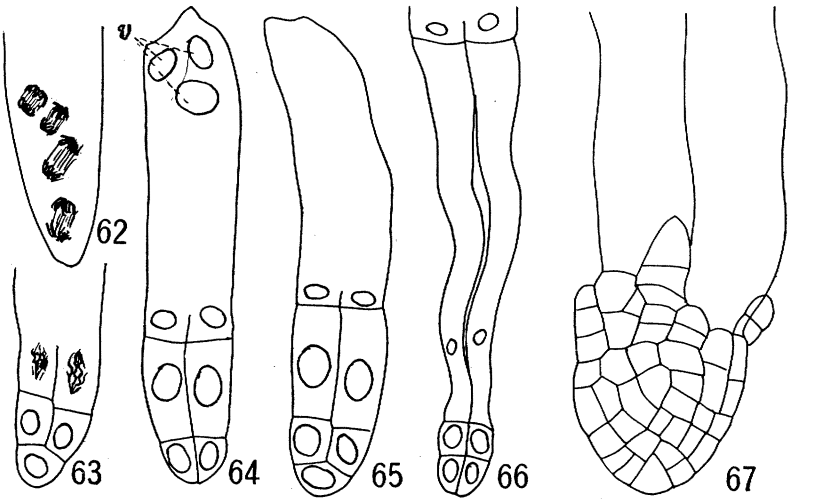
In the Cupressineae, the proembryo also resembles that of Pinus except for the unequal tier organization. In Juniperus (figs. 52–61), which represents the condition nearest to Pinus, the proembryo seems to organize



FIGS. 52–61. Stages in embryogeny of Juniperus. FIGS. 53 and 55. *Juniperus communis* var. *depressa*, × 150, after Nichols (30). FIGS. 52, 54 and 56. *J. communis*, after Noren (31). FIG. 58. Same, after Hofmeister (17). FIGS. 57, 59–61. *J. virginiana*, after Strasburger (40). Figure 59 shows the many embryos arising from an archegonial complex, multiplied further by cleavage polyembryony, × 75. Figures 56–58 indicate that an uneven tier arrangement (shown in figures 52 and 54) is no hindrance to cleavage embryony. Figure 53 shows that embryo initials may organize *after* the first walls form, and figures 60 and 61 (× 185) show the apical cell.

with the usual embryo initials in several tiers and, above, some inactive nuclei that abort (17, 30, 31, 40). The walled cells below these aborting nuclei function as suspensors, while the lowest group of embryo initials becomes organized into embryos that separate. Figures 56–59 show that cleavage polyembryony is found, and figures 59–61 that the apical cell stage is probably the normal condition.

Thuja (figs. 62–67) is a step higher, for here cleavage polyembryony does not occur normally. I find from my own studies[4] of this species that it occurs occasionally, probably coming from such embryos as shown in figures 64 and 66 by Land (21). In most cases, one of the embryo initials,



FIGS. 62–67. Stages in embryogeny of *Thuja occidentalis*. Figures 62–66, after Land (21), × 300. FIG. 87. Later stage showing apical cell of embryo, × 225, after Strasburger (40).

such as the lowest of figure 65, has a more favorable position and gives rise to the embryos, the others only elongating to form the suspensors.

Whether only the terminal embryo initial functions or several of them, an apical cell stage is always found; a very conspicuous feature in Thuja (fig. 67). From my own studies[4] of *Thuja occidentalis*, I can also state that rosette embryos are found in a significant number of cases, but they are not found in *Thuja orientalis*.

Tetraclinis probably represents a more advanced condition than Thuja, for it appears that only the terminal embryo initial functions. Saxton (36) reported no splitting embryos, but found that several tiers of the proembryo may contribute to the suspensor. However, the existence of cleavage polyembryony may easily have been overlooked.

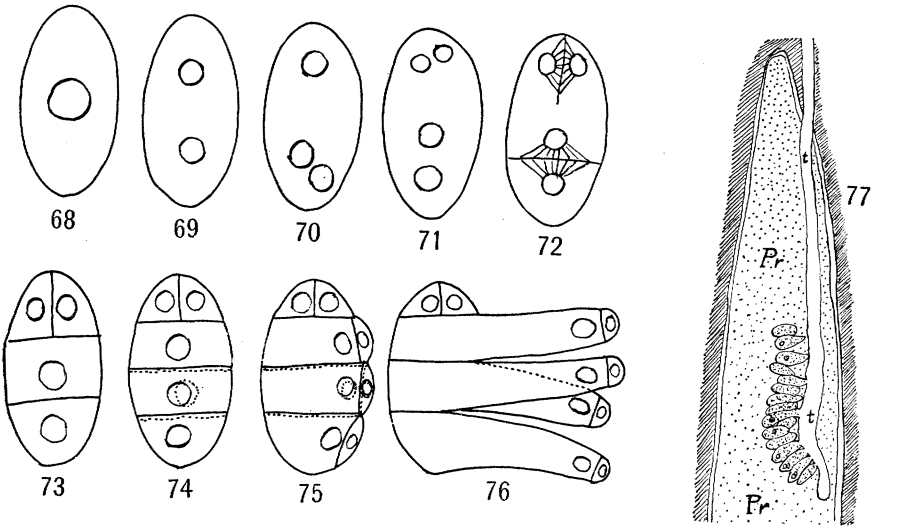
In Libocedrus, which was studied by Lawson (26), the embryogeny is also too little known to justify any conclusions, and the embryogeny of both Tetraclinis and Libocedrus should be reinvestigated to clarify some of these points.

The Taxodineae and Cupressineae appear to be very similar on the basis of embryogeny, both groups showing an evolution from cleavage polyembryony to simple polyembryony, but our knowledge of both of these groups is at present very unsatisfactory.

[4] Unpublished work.

Actinostrobus and Callistris

The Actinostrobus and Callitris type of embryo development (figs. 68–76) has certainly been derived from one of the types already described, probably from that of the Cupressineae. In Actinostrobus (35), the first walls appear earlier, between the two- and the four-nucleate stages. The proembryo tissue fills the entire egg, and the separate embryo initials are not organized from the first walled cells, as in Pinus, but after one of the



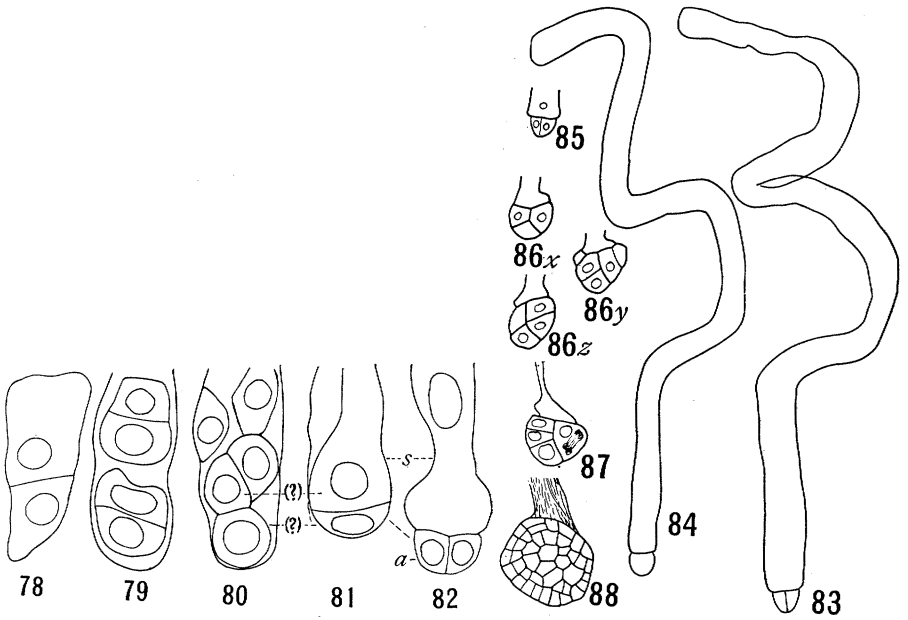
Figs. 68–76. Diagrams to represent proembryo of Actinostrobus. Callitris, and probably Widderingtonia, are very similar. After Saxton (35). Fig. 77. Gametophytes of Callitris, showing large lateral archegonial complex, in its relation to the pollen tube (*t*). After Saxton (34).

succeeding divisions. Two of the first walled cells undergo no further development, and four embryo initials are organized in the remainder of the egg. Figure 77 shows the relation of the gametophytes and the lateral archegonial complex in Callitris. From this it will be seen that the embryos which grow out laterally from these archegonia penetrate the gametophyte in the usual manner. Saxton, to whom we are indebted for our knowledge of this interesting embryogeny, states that Callitris (34) is very similar, while Widderingtonia (32, 33) is transitional between this and some of the Cupressineae. Cleavage polyembryony is a constant feature, and in all probability the apical cell is also found in the first stages of the embryo.

Widderingtonia, Actinostrobus, and Callitris have been placed in a separate sub-family, the Callitroideae, by Saxton (37), largely on the basis of the gametophytic and embryonic characters, and it seems that there is good ground for including these in a group distinct from the other Cupressineae.

SEQUOIA

In the proembryo of Sequoia (23) the tendency to an early wall formation is carried a step further than in the forms previously described, for a wall is laid down after the first division of the egg nucleus (fig. 77). Each of these proembryo cells rounds off more or less, and the cells resulting from



FIGS. 78–82. Stages in the embryogeny of *Sequoia sempervirens*, × 250, after Lawson (23).

FIGS. 83–88. Later stages in the embryogeny of Sequoia, after Arnoldi (1); figure 87 showing an apical cell. FIGS. 86*x*, *y*, *z*, series of three sections through an eight-celled embryo showing apical cell, after Shaw (38).

the next division are similarly dissociated. According to my interpretation, these cells shown in figures 79 and 80 are separately organized embryo initials, all but the lowest of which abort; at least their further development has not been observed. The lowest cell of figure 79 (or the lowest of figure 80, a point not made clear in the account by Lawson) gives rise to the embryo shown in the succeeding figures, the suspensor cell being the first cell cut off from the embryo initial. This earlier wall formation, the proembryonic tissue filling the entire egg, and the suppression of cleavage polyembryony by the abortion of embryo initials, are some of the advanced embryo characters illustrated by Sequoia.

There is probably at least a short apical cell stage in Sequoia, according to the figures of Shaw (38) and Arnoldi (1), shown here in figures 83–88.

EPHEDRA

I cannot refrain from including a mention of the Gnetales in this discussion, for these have polyembryony, much as have the Coniferales. In the embryo of Ephedra (*22*, 40), for example, cleavage polyembryony is the regular occurrence. The free nuclei which are concerned in the organization of the embryo initials do not all descend to the bottom of the egg, as in Pinus, but remain in comparative independence of each other, and this condition of cleavage polyembryony is very striking. Judging from the published accounts and figures there is not much probability that an apical cell stage is to be found, and this would furnish us an instance in which the apical cell is eliminated and cleavage polyembryony is retained. It seems to me that this feature of cleavage polyembryony very definitely marks the Gnetales as having been derived from the conifers rather than from the cycads.

There is perhaps no better way for me to summarize these many comparisons of embryo development made during the course of this discussion, than to use the method of a diagram, as was done for the Abietineae. Such a scheme of phylogeny represents only the present status of our knowledge concerning embryogeny and is not intended to be accurate to the last details, for many of these details have been too inadequately described. It doubtless represents fairly accurately the affinities of the larger groups.

In this diagram (fig. 89), the ranges of the various embryonic features which have been discussed are plotted in light lines, while the (probable) phylogenetic connections suggested by embryogeny are shown in the heavy lines. The range of the apical cell (*A*), and cleavage polyembryony (*Cl.p*) are marked with some uncertainties, but in general it appears that the latter is more limited in its range than is the apical cell. The archegonial complex is found in all forms surrounded by the circle *Cx*. Rosette embryos are not plotted, but the forms having rosette cells are marked as follows: *R*, rosette cells; (*R*), occasional rosette cells; *R-em*, rosette cells that develop embryos; *R-(em)*, rosette cells that occasionally give rise to embryos, etc. The rosette embryos are therefore also primitive features that soon vanish, followed by the disappearance of the rosette cells, which are the last vestiges of cleavage polyembryony. The embryo cap (*Emb.cap*) found in Araucaria and Agathis is an advanced feature found also in the forms somewhat involved in the evolution of the Araucarineae.

It will be found that in general the cotyledon number decreases as we proceed upward in every direction away from Pinus. Though the number of cotyledons is somewhat variable throughout, this number is reduced sooner or later among conifers (3) to the limit of three or two.

I have included the range of one well-known taxonomic feature in this diagram, namely, the spur shoot (*Sp.S*). Sciadopitys with its double needles

in the axil of a bract has doubtless also the morphological equivalent of the spur shoot. If the phylloclad, which occupies the same position on the stem, could be looked upon as the morphological equivalent of this dwarf branch, we should have the dimorphic branches represented in a

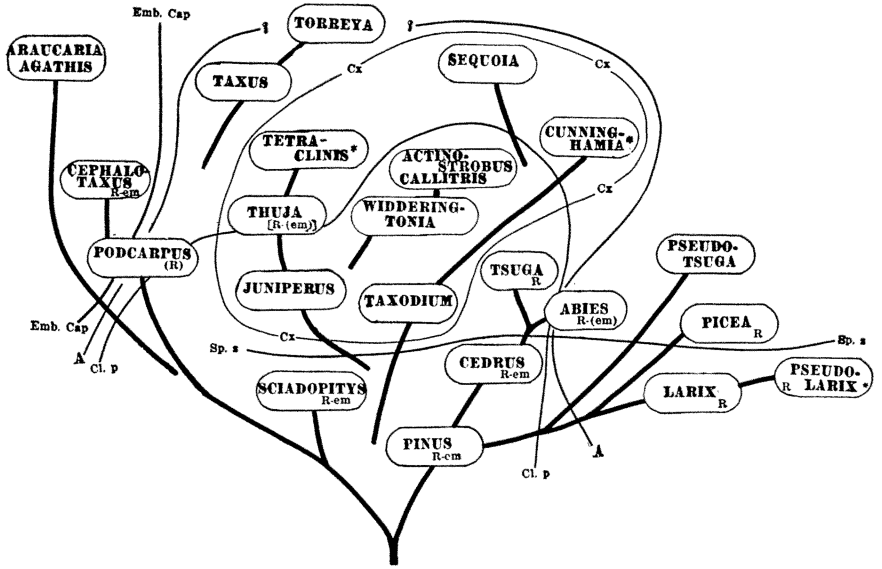


FIG. 89. Diagram representing the affinities of the Coniferales as suggested by embryogeny. *A*, probable range of distribution of apical cell in early embryo; *Cl.p*, probable range of cleavage polyembryony; *Emb.cap*, range of distribution of embryo cap; *Cx*, distribution of archegonial complex; *R*, rosette cells present; *R-em*, rosette cells that usually give rise to rosette embryos; *R-(em)*, rosette cells and rosette embryos occasional only; *Sp.s*, range of distribution of spur shoot. Position of forms marked with asterisk (*) is doubtful.

member of the Podocarpineae. Jeffrey (20) looks upon the spur shoot as a primitive feature, and it would appear at least that this dwarf branch is a character present in the most primitive representatives of more than one line of evolution.

Unfortunately the embryogeny of a large number of conifers is not known, and many forms that have been studied have been described in such a way that we are uncertain of the features with which we are most concerned in the present discussion. Enough has been described to indicate that with some such organization of the facts as here presented, embryogeny must occupy a much more important place in the study of comparative morphology than has generally been conceded.

The origin of cleavage polyembryony, a very interesting question, has not been shown in any living conifer thus far described. A theory to account for the possible origin of this condition has been formulated and is to be published at another time.

SUMMARY

This brief review has summarized, or taken into account, practically all of the published work on the embryogeny of conifers in the proembryo and early embryo stages. It is shown how the known facts may be harmonized under the conception that the Coniferales have been derived from forms with cleavage polyembryony, and that this feature tends to be more or less eliminated as we pass from the lower to the higher forms. The apical cell, cleavage polyembryony, rosette embryos, rosette cells, and the direct organization of embryo initials from the free nuclei of the proembryo, are regarded as primitive features, while the organization of embryo initials after walls form in the proembryo, a proembryo that fills the entire egg with cells, the archegonial complex, the embryo cap, and the return to simple polyembryony, must be regarded as advanced or specialized features. Transitional conditions may also be recognized, and some of the vestigial characters, in disappearing by gradual steps, make this interpretation of the direction of evolution from cleavage polyembryony back to simple polyembryony very certain. Embryogeny must occupy a much more important place in the comparative morphology of conifers than has generally been conceded.

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LITERATURE CITED

Additional citations on the embryogeny of Abietineae with review may be found in the writer's paper on Abietineae (4).

1. **Arnoldi, W.** Beiträge etc. V. Weitere Untersuchungen der Embryogenie in der Familie der Sequoiaceen. Bull. Soc. Imp. Nat. Moscou, n.s. **14**: 449–476. pls. 7–8. 1900.
2. **Buchholz, J. T.** Suspensor and early embryo of Pinus. Bot. Gaz. **66**: 185–228. pls. 6–10, figs. 3. 1918.
3. ——. Studies concerning the evolutionary status of polycotyledony. Amer. Journ. Bot. **6**: 106–119. figs. 25. 1919.
4. ——. Polyembryony among Abietineae. Bot. Gaz. **69**: 153–167. figs. 15. 1920.
5. **Burlingame, L. L.** The morphology of *Araucaria brasiliensis* III. Fertilization, the embryo, and the seed. Bot. Gaz. **59**: 1–39. pls. 1–3. 1915.
6. **Chamberlain, C. J.** The living cycads. Chicago, 1919.
7. **Chrysler, M. A.** The medullary rays of Cedrus. Bot. Gaz. **59**: 387–396. figs. 7. 1915.
8. **Coker, W. C.** Notes on the gametophytes and embryo of Podocarpus. Bot. Gaz. **33**: 89–107. pls. 5–7. 1902.
9. ——. On the gametophytes and embryo of Taxodium. Bot. Gaz. **36**: 1–27, 114–140. pls. 1–2. 1903.
10. ——. Fertilization and embryogeny of *Cephalotaxus Fortunei*. Bot. Gaz. **43**: 1–10. pl. 1, figs. 5. 1907.
11. **Cook, Mel T.** Polyembryony in Ginkgo. Bot. Gaz. **36**: 142. 1903.
12. **Coulter, J. M., and Chamberlain, C. J.** Morphology of Angiosperms. New York, 1903.
13. ——, ——. Morphology of Gymnosperms. Chicago, 1910 and 1917.
14. ——, **and Land, W. J. G.** Gametophytes and embryo of *Torreya taxifolia*. Bot. Gaz. **39**: 161–178. pls. A, 1–3. 1905.

15. **Eames, A. J.** The morphology of *Agathis australis*. Annals of Botany **27**: 1–38. pls. 1–4. 1913.
16. **Engler, A., and Gilg, E.** Syllabus der Pflanzenfamilien. Berlin, 1912.
17. **Hofmeister, W.** Higher cryptogamia and fructification of the Coniferae. Translation, Ray Society, London, 1862.
18. **Jäger, L.** Beiträge zur Kenntniss der Endospermbildung und zur Embryologie von *Taxus baccata*. Flora **86**: 241–288. pls. 15–19. 1899.
19. **Jeffrey, E. C.** The comparative anatomy of Coniferales II. The Abietineae. Mem. Boston Soc. Nat. Hist. **6**: 1–37. pls. 1–7. 1904.
20. ——. The anatomy of woody plants. Chicago, 1917.
21. **Land, W. J. G.** A morphological study of Thuja. Bot. Gaz. **34**: 249–259. pls. 6–8. 1902.
22. ——. Fertilization and embryogeny in *Ephedra trifurca*. Bot. Gaz. **44**: 273–292. pls. 20–22. 1907.
23. **Lawson, A. A.** The gametophyte, archegonia, fertilization, and embryo of *Sequoia sempervirens*. Annals of Botany **18**: 1–28. pls. 1–4. 1904.
24. ——. The gametophyte, fertilization and embryo of *Cryptomeria japonica*. Annals of Botany **18**: 417–444. pls. 27–30. 1904.
25. ——. The gametophytes, fertilization, and embryo of *Cephalotaxus drupacea*. Annals of Botany **21**: 1–23. pls. 1–4. 1907.
26. ——. The gametophytes and embryo of the Cupressineae with special reference to *Libocedrus decurrens*. Annals of Botany **21**: 281–301. pls. 24–26. 1907.
27. ——. The gametophytes and embryo of *Sciadopitys verticillata*. Annals of Botany **24**: 403–421. pls. 29–31. 1910.
28. **Miyake, K.** The development of the gametophytes and embryogeny of *Cunninghamia sinensis*. Beih. Bot. Centralbl. **27**: 1–25. pls. 1–5, figs. 2. 1910.
29. ——, **and Yasui, K.** On the gametophytes and embryo of *Pseudolarix*. Annals of Botany **25**: 639–647. pl. 48. 1911.
30. **Nichols, G. E.** A morphological study of *Juniperus communis* var. *depressa*. Beih. Bot. Centralbl. **25**: 201–241. pls. 8–17, figs. 4. 1910.
31. **Noren, C. O.** Zur Entwickelungsgeschichte des *Juniperus communis*. Upsala Universitets Arsskrift 1907. pp. 64. pls. 4.
32. **Saxton, W. T.** Preliminary account of the development of the ovule, gametophytes, and embryo of *Widderingtonia cupressoides* Endl. Bot. Gaz. **48**: 161–178. pl. 11, figs. 3. 1909.
33. ——. Contributions to the life history of *Widderingtonia cupressoides*. Bot. Gaz. **50**: 30–48. pls. 1–3. 1910.
34. ——. Contributions to the life history of Callitris. Annals of Botany **24**: 557–569. pls. 45, 46. 1910.
35. ——. Contributions to the life history of *Actinostrobus pyramidalis*, Miq. Annals of Botany **27**: 321–345. pls. 25–28. 1913.
36. ——. Contributions to the life history of *Tetraclinis articulata* Masters, with some notes on the phylogeny of the Cupressineae and Callitroideae. Annals of Botany **27**: 577–603. pls. 44–46, figs. 9. 1913.
37. ——. The classification of conifers. New Phytologist **12**: 242–262. fig. 1. 1913.
38. **Shaw, W. R.** Contribution to the life history of Sequoia. Bot. Gaz. **21**: 332–339. pl. 24. 1896.
39. **Sinnott, E. W.** The morphology of the reproductive structures of the Podocarpineae. Annals of Botany **27**: 39–82. pls. 5–9, figs. 9. 1913.
40. **Strasburger, E.** Die Coniferen und die Gnetaceen. Jena, 1872.
41. ——. Die Angiospermen und die Gymnospermen. Jena, 1897.